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Brief communication (Original)

Serum levels of 25-OH vitamin D, folic acid and testosterone in patients with breast cancer: a case control study

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Background: Breast cancer is the most common cancer in women. To study factors causing breast cancer, various epidemiological and experimental studies are being conducted. A relation may exist between vitamin D, folic acid, testosterone, and various neoplasms.

Objectives: Detect the levels of vitamin D, folic acid, and testosterone in breast cancer of patients and healthy subjects, and determine the relation of the levels of vitamin D, folic acid, and testosterone with tumor histopathology, stage, and receptor status.

Methods: Eighty patients diagnosed with breast cancer and 20 controls (healthy volunteers) were enrolled in the study. Serum samples were collected from the patients and the controls, and examined using Roche E-170 with its own special kit.

Results: Folic acid levels were higher in the patient group and the difference was statistically significant (p=0.044). The levels of testosterone were lower in post-menopausal patients (p=0 .018). In stage I-II (early-stage) patients, vitamin D levels were higher, while in the stage III-IV (advanced-stage) patients, the levels were lower (p=0.048).

Conclusions: Low vitamin D levels may be related to poor prognosis. Low levels of folic acid were detected in the control group. Low levels of folic acid but high vitamin D and testosterone levels may be protective against breast cancer.

Keywords: Breast neoplasms, folic acid, serum, testosterone, vitamin D

Breast cancer is the most frequent cancer in women. To elucidate the risks of breast cancer, various epidemiological and experimental studies are being carried out in vitamins and endogen sex hormones.

Vitamin D is a potent regulator of the cell growth and differentiation. It has effects on cell death, invasion of the tumor and angiogenesis [1]. Deficiency of vitamin D may increase the risk of some neoplasms [2, 3]. Since 1,25-dihydroxyvitamin D inhibits the proliferation of human melanoma cells *in vitro* [1], studies are focused on the anti-proliferative effects of the vitamin D. Folate plays an important role in methylation reactions and one-carbon transferring for the synthesis of nucleotides [4]. It also plays role in cancers of certain organs such as colorectal, lung, pancreas, esophagus, stomach, cervix, breast, and leukemia [5, 6]. It has been predicted that low levels of folic acid relate to the development of cancer. Graham et al. [7] detected inverse correlation between folate intake and breast cancer.

Sex steroids play role in the etiology of breast cancer. It has been thought that androgens increase the risk of breast cancer. Normal and malign breast epithelial cells include androgen receptors [8]. Both androgens and androgen precursors have effects on the growth of breast and malign breast cells [9].

In this study, we detected the levels of vitamin D, folic acid, and testosterone in breast cancer patient to evaluate the difference between the levels in patients and healthy subjects. We tried to determine the relation

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between the levels of vitamin D, folic acid, and testosterone with tumor histopathology, stage, and receptor status.

Materials and methods

Eighty patients diagnosed with breast cancer and 20 controls (age matched) were enrolled in this case control trial. Patients were selected from those who sought care from the Medical Oncology Clinic, Medical Faculty, Karadeniz Technical University. Non-metastatic patients were enrolled post-operatively, before the initiation of systemic adjuvant therapy and metastatic patients were enrolled before the systemic therapy.

Control group was selected from the healthy volunteers. Patients and controls consented to be part of the study. This study was approved by the Ethics Committee of Medical Faculty, Karadeniz Technical University. The diagnosis and the date of diagnosis were determined based on their pathology report. The patients were staged according to the American Joint Committee [10].

Serum samples were collected before the chemotherapy and stored at -20 C until the initiation of the study. The patients' and control groups' serum samples were thawed just before the biochemical study, which were examined using Roche E-170 (Mannheim, Germany) with its own special kit. References values were Roche 11.1-42.9 ng/mL for 25-OH vitamin D3, 4.6-18.7 ng/mL for folate Roche, and 0.06-0.82 ng/mL for testosterone women Roche.

Statistical analyses

The conformance of data obtained by measurement to normal distribution was investigated in each group by Kolmogorov Smirnov test. The comparisons of the variables conforming to normal distribution were made by Student t-test between the parameters and the means values of vitamin D, folic acid, and testosterone. As for the comparisons of the variables not conforming to normal distribution, MannWhitney U test was used. The data obtained by measurement were expressed as means standard deviation (SD). The significance level was $p < 0.05$.

Results

Eighty patients diagnosed with breast cancer and 20 controls were included in the trial (100 participants). All patients and controls were female. There was no statistically significant difference between the patients and the control group with respect to age (p=0.868). 25-OH vitamin D3 levels were evaluated in patients and controls. There was no statistically significant difference between the groups (p=0.530). Testosterone levels were evaluated, but no statistically significant was detected (p=0.16). Folic acid levels were higher in the patient group, and the difference was significant (p=0.044) as shown in **Table 1**.

There were 21 pre-menopausal and 55 post-menopausal women. Other four patients' menopause status was not recorded. The mean testosterone level was 0.34±0.17 ng/mL in premenopausal patients, and 0.25±0.13 ng/mL in postmenopausal patients. The levels of testosterone were lower in postmenopausal patients (p=0.018). The difference was not statistically significant with respect to the levels of vitamin D and folic acid (p= 0.520 and p=0.832, respectively).

Sixteen patients were less than 45 years old, while 64 patients were greater than 45 years old. Mean testosterone level was higher in patients with age less than 45 years, and the difference was statistically significant between the groups (0.37±0.19 ng/mL *vs.* 0.25±0.13 ng/mL, p=0.008). With respect to level of vitamin D and folic acid, no statistically difference was detected (p=0.595 and p=0.324, respectively).

Table 1. Mean levels of Vitamin D, folic acid and testosterone in breast cancer patients

	Patients (mean±SD)	Controls (mean±SD)	P-value
Age (years)	53.6±10.7	53.2±10.8	0.868
25(OH) vitamin D3 (ng/mL)	23.80±14.60	25.90±7.30	0.530
Folic acid (ng/mL)	11.40±11.30	8.70±1.90	0.044*
Testosterone (ng/mL)	0.280±0.165	0.336±0.178	0.160

*Statistically significant

The patients were classified according to the histological subtype as ductal and non-ductal carcinoma. Between these groups, the levels of vitamin D, testosterone and folic acid were not statistically significantly different (p=0.976, p=0.941, and p=0.308, respectively). No relation was detected between the other parameters (tumor diameter, lymph node status, tumor grade, and lymphovascular invasion), vitamin D, testosterone, and folic acid levels. In stage I-II (early- stage) patients, vitamin D levels were higher, while in the stage III-IV (advanced-stage) patients the levels were lower (median: 35.43 and 26.47, p=0.048). With respect to the levels of the folic acid and testosterone, statistically significant difference was not detected (p=0.686 and p=0.989, respectively).

Estrogen receptor (ER), progesterone receptor (PR) status, and CerbB2 (known as human epidermal growth factor receptor 2) status were evaluated. In 37 patients, ER and PR were both together positive, and mean testosterone level was 0.31±0.18 ng/mL. ER+/PR-, ER-/PR+, ER-/PR- patients were classified as the other group, and mean testosterone level was 0.23±0.11 ng/mL. The difference was statistically significant between the groups (p=0.034). Vitamin D and folic acid levels were not statistically significant according to the ER and PR status. There was no statistically significant difference in cerbB2 status (**Table 2**).

Table 2. Relationship between variables and mean levels of vitamin D, folic acid, and testosterone in breast cancer patients

Variables	Vitamin D (ng/mL) (mean SD)	P-value	Folic acid (ng/mL) (mean SD)	P- value	Testosterone (ng/mL) (mean SD)	P-value
Age (years)						
<45	21.6 0±5.50	0.595	10.52±4.26	0.324	0.38±0.20	0.008*
≥45	24.35±16.56		11.97±12.70		0.26±0.13	
Menopause status						
Pre-menopause	21.65±5.70	0.520	11.68±10.55	0.832	0.34±0.17	0.018*
Post-menopause	24.62±17.58		11.661±2.09		0.25±0.13	
Tumor size						
<5 cm	25.89±17.67	0.357	13.09±13.68	0.733	0.28±0.15	0.393
≥5 cm	19.38±3.56		9.24±3.70		0.32±0.15	
Lymph node status						
Negative	25.22±16.07	0.236	10.17±9.43	0.366	0.25±0.11	0.224
Positive	24.05±15.80		13.19±13.50		0.30±0.18	
Stage						
I-II	27.07±17.40	0.048*	14.12±15.63	0.686	0.27±0.15	0.989
III-IV	22.09±14.21		10.48±8.04		0.27±0.16	
Tumor grade						
I-II	24.06±15.44	0.374	10.68±10.56	0.480	0.29±0.17	0.415
III	24.70±18.05		13.30±13.66		0.26±0.14	
Histological type						
Ductal	23.44±13.66	0.976	11.65±11.36	0.308	0.27±0.16	0.941
Non-ductal	25.79±19.53		10.94±13.56		0.28±0.13	
Lymphovascular invasion						
Negative	22.64±10.75	0.817	11.83±12.52	0.787	0.26±0.19	0.665
Positive	25.89±19.32		11.87±12.15		0.29±0.13	
ER						
Positive	22.42±12.80	0.367	9.98±8.72	0.441	0.30±0.17	0.084
Negative	25.02±13.45		15.15±16.59		0.22±0.13	
PR						
Positive	24.21±14.33	0.288	10.68±9.86	0.943	0.31±0.18	0.062
Negative	21.78±11.06		12.13±13.17		0.24±0.12	
CerbB2						
Positive	22.02±13.91	0.216	13.69±16.28	0.779	0.26±0.16	0.521
Negative	23 48±12.71		10.42±8.93		0.29±0.16	

* Statistically significant. ER=estrogen receptor, PR=progesterone receptor. CerbB2=human epidermal growth factor receptor 2

Discussion

In the present study, we detected that vitamin D levels were lower in breast cancer patients compared with the controls, but the difference was not statistically significant. According to the stage, patients with advanced stage had low vitamin D levels. This suggests that low vitamin D levels might be associated with the prognosis. It was shown *in vitro* that vitamin D3 has inhibitor effects on breast cancer cells [11-13], inducing the apopitosis in this cell lines [1]. The protective effect of vitamin D3 on developing breast cancer was detected in NHANES 1 study [14]. Palmieri et al. [15] measured 25(OH) vitamin D levels in patients with early and advanced stage breast cancer, and demonstrated that the levels were statistically significantly low in patients with advanced stage. This finding indicates that *in vitro* effects are a reflection *of in vivo* ones. Mayer et al. [16] investigated serum 1,25-dihydroxyvitamin D in breast cancer patient with bone metastases, and showed that serum vitamin D levels fell as diseases progress. Our findings support that the hypothesis of high levels of vitamin D may inhibit the growth and progression of the breast cancer cells.

Androgens play an important role in the carcinogenesis of the breast cancer. According to Liao and Dickson [17], androgens directly or indirectly affect the cell growth and self-proliferation or by turning into estrogen. In the EPIC study, Kaaks et al. [9] detected the relation between high serum androgens and high risk of breast cancer. In our study, testosterone levels were lower in the patient group, but the difference was not significant between the patients and the controls. In post-menopausal patients with age 45 years and above, testosterone levels were lower. This lowering of testosterone levels might be due to a decrease in androgens synthesis or an increase in converting estrogens for stimulating the malignant cells. In our patients with ER (or PR positivity) only and in the ER+/PR+ group, high levels of testosterone was detected. In a study by Eliassen et al. [18], the increased risk of ER+/PR+ breast cancer was detected in patients with high levels of total, free testosterone and andronostenedion. Our results support these findings. Although we evaluated the levels of total testosterone in our study, we did not separate the levels according to the menstrual cycle in the pre-menopausal patients.

In animal and human studies, folate often modulates the carcinogenesis, as shown in the case of colorectal cancers [5]. In fact, folate plays an important role in methylation reactions and synthesis of the nucleotides responsible for the carcinogenesis. In our study, the folic acid levels in cancer patients were higher than the controls. On the other hand, in a case-control study by Graham et al. [7], negative relation was reported between the risk of breast cancer and dietary folate intake. However, in an animal study by Baggot et al. [19], folate intake accelerated the early development of mammary cancer, and folate deficiency could protect the rats from the development of mammary cancer. The effect of folic acid on breast cancer is not clear, but our measured high folic acid levels agree with the results of animal study.

In conclusion, low vitamin D levels may be related with the poor prognosis. Low levels of folic acid but high vitamin D and testosterone levels may be protective against breast cancer. The conclusion needs to be validated with more studies.

The authors have no conflict of interest to declare.

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